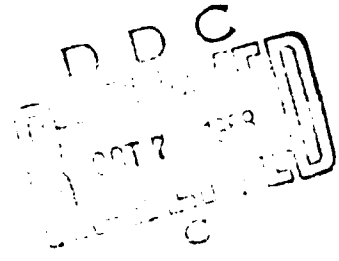
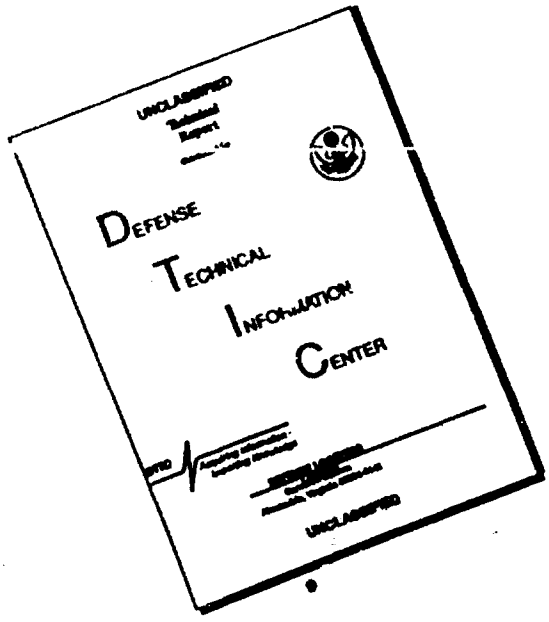
TRANSLATION NO. 3246

DATE: June 1967



DEPARTMENT OF THE ARMY
Fort Detrick
Frederick, Maryland

DISCLAIMER NOTICE



THIS DOCUMENT IS BEST QUALITY AVAILABLE. THE COPY FURNISHED TO DTIC CONTAINED A SIGNIFICANT NUMBER OF PAGES WHICH DO NOT REPRODUCE LEGIBLY.

MORPHOLOGY OF IMMUNOGENESIS DURING EXPERIMENTAL ANTHRAX VACCINATION

[Following is the translation of an article by B. S. G[illegible]sman and [illegible]. V. Mi[illegible], State Control Institute for Medical-Biological Preparations imeni [illegible] and the Institute of Human Morphology, [illegible] in the Russian-language periodical [illegible] Immunobiologii (Journal of Microbiology, Epidemiology and Immunobiology) No 12, 1966, pages 58--63. It was submitted in March 1966. Translation performed by Sp/7 Charles T. Ostertag, Jr.]

A vaccine against anthrax was proposed by Pasteur in 1880. In Russia immunization against anthrax was carried out for the first time by Tsenkovskiy in 1883. At the present time immunization against anthrax is carried out with the STI-1 vaccine strain. This was obtained by Ginsburg (1946) and possesses high immunogenic properties.

The aim of the present investigation was the study of the morphological reactions, developing in experimental animals following vaccination with the STI-1 and 34-F_2 (American) strains. The experiments were set up on 42 rabbits and 82 guinea pigs. Twelve rabbits and 13 pigs served as a control.

The rabbits were given the vaccine strains in amounts of 40 and 160 million spores. The period of observation was from 24 hours up to 2 months. The pigs were injected with these 2 strains in doses of 20 million spores. The experimental animals were sacrificed (decapitation) in 1, 2, 7, 14, 30, 45, 60, 90, 120, 150, 180, 210 days after administration of the vaccine.

Histological investigations were made on the lymph nodes, spleen, bone marrow, internal organs and tissue from the site of administration of the vaccine. Fragments were fixed in acetone in the cold, sealed in paraffin-celloidin, and microscopic sections (5 microns thick) stained with hematozylin, azan, by the Brash method for RNA, for glycogen, and in addition to this cardiac muscle was stained by the Selye method.

Upon autopsy of rabbits on the 2nd day after vaccination, edema was detected at the site of administration. It was especially sharply expressed following inoculation with the 34-F_2 strain (distributed throughout the subcutaneous tissue of the hip; in some cases there was hemorrhaging and the formation of abscesses). In the spleen there was hyperplasia of the follicles, and in a number of cases the liver was flaccid.

During the histological investigation during the first days following the administration of the STI-1 vaccine strain, at the site of injection on the rabbits we observed a small edema of the subcutaneous tissue, dilatation of the blood vessels and infiltration with segmentonuclear leukocytes. Following staining for RNA, pyroninophilic cells were not found. By the 4th day the symptoms of edema and inflammation were increased and remained sharply expressed up to the 14th day. During these periods the cellular composition changed and during staining for RNA it was possible to expose a number of mature and immature plasmatic cells (figure 1).

Starting with the 14th day the formation of granulation tissue began at the site of administration of the vaccine. By the 24th day the inflammatory changes abated, but as before a large quantity of pyroninophilic cells were detected. By the 45th day pathological changes were absent and following staining for RNA individual plasmatic cells appeared. When the 34-F_2 vaccine strain was administered, more expressed local changes were noted (strong edema by the 4th day, sharply expressed inflammatory changes with necrosis of the muscle fibers of the subcutaneous tissue) with the formation of abscesses and the appearance of pyroninophilic cells in the first days following administration.

In the histological investigation of the regional lymph node 24 hours after inoculation with strains being studied, we detected hyperplasia of the embryonic centers of the secondary nodules, some dilatation of the sinuses, swelling of the endothelium, and proliferation of the reticular cells. In many of these, figures of mitosis were distinguished and there was a small number of plasmoblasts. All the changes increased by the 14th day, and then gradually abated, but on the 60th day (end of observation) a considerable number of cells of a plasmatic nature were still revealed.

Following the administration of the STI-1 vaccine strain, analogous changes appeared in the axillary lymph node (remote) somewhat later - from the 14th to the 24th days. By the end of the observations, that is, 2 months, the condition of the lymph node had normalized. Following the administration of the 34-F_2 vaccine the described changes were more temporary - normalization of the lymph node set in by the 14th day.

In the spleen, following the administration of both vaccine strains, we detected similar microscopic changes, developing by the 4th day after inoculation of the vaccine and expressed in a minor plethora, dilatation of the sinuses, hyperplasia in the reproduction centers of the follicles and increase in the number of plasmoblasts both in the center of the follicles and in the pulp of the spleen. Pictures of mitosis were apparent in the reticular cells. A macrophagal reaction appeared. The most expressed changes were observed on the 24th day.

By the end of the observation (2 months) the changes had abated, but all of the plasmatic cells in the vaccinated animals were larger than in the control animals.

In the bone marrow of the test rabbits we detected processes of hyperplasia with plasmatization of the cellular make-up (figure 2), which began with the 7th day after administration of the vaccine and were detected up to the end of the investigation (2 months).

Clear immunomorphological changes were developed in the lungs. They were expressed in plasmatization of the lymphoid follicles, desquamation of the alveolocytes and focal proliferation of the cellular elements in the interalveolar septa with the appearance of plasmatic cells in them (fig 3). These symptoms reached maximum intensity on the 14th day after administration of the vaccine.

In the liver of the test rabbits from the 1st through the 14th days after administration of the vaccine strains we observed a minor plethora, dilatation of the lymph fissures and a large quantity of glycogen in the hepatic cells.

Changes in the parietal and valvular endocardium were limited to swelling of its endothelial layer, monor mucoid edema of the heart valves, and insignificant cellular proliferation. In the cardiac muscle, when stained according to Selye, there was exposed a various degree of fuchsinophilic degeneration of the fibers (figure 4), and small cellular infiltrates, which included individual plasmatic cells. These changes were most expressed 2 weeks after administration of the vaccine and by the end of the investigation had abated (2 months).

In the kidney, mainly following the administration of the 34-F_2 strain, we detected monor plethora and swelling of the epithelium of the convoluted tubules.

During the autopsy of the vaccinated guinea pigs we observed the same picture as in the rabbits. During the histological investigation of tissue from the site where the vaccine strains were administered, and also the spleen, lymph nodes and bone marrow we detected changes analogous to those described in the rabbits, but differing in the periods of appearance and disappearance. Thus, in the regional lymph node, spleen and bone marrow the symptoms of plasmatization, mitosis and the macrophagal reaction were observed for the course of 5 months, and during this period they were most expressed in the bone marrow.

It was established by statistical processing that the weight of the spleen in the experimental animals reached a maximum on the 14th day after vaccination, then the curve of their weight was maintained at approximately the same level up to the 30th day and gradually lowered, coming close to the initial value in the 5th month (figure 5).

In this manner, for the course of 7 months we followed the dynamics of the immunogenesis process in rabbits and guinea pigs, immunized with anthrax vaccine. All the lymphoid organs were involved in the process of immunogenesis, but in the remote lymph node and spleen the changes were expressed considerably more weakly than in the regional. This conforms fully with data from the literature. McMaster and Hudack (1935) showed the primary accumulation of antibodies in the regional lymph node. Gurvich and Shumakova (1960) consider that the process of antibody production may be limited, that is, only the regional lymph node is included following the administration of soluble antigen, or it is distributed throughout the entire lymphatic system following immunization with corpuscular antigen. An oil emulsion, used during vaccination against anthrax for the purpose of deposition of the soluble antigen, imparts to it certain properties of the corpuscular antigen which stimulate the involvement of the remote lymph nodes and spleen in the process. There are reports in the literature (Gavrilov, 1929) on the particular role of the spleen during vaccination against anthrax. It has been established experimentally that a splenectomy disrupts the immune condition of rabbits which have been vaccinated against anthrax, while following the administration of other antigens a splenectomy does not inhibit the production of antibodies (Myerson et al., 1957).

Proliferation processes, detected in the interalveolar septa of the lungs, had a similarity with morphological changes during interstitial pneumonia. Following vaccination they represent the nonspecific immunomorphological reaction of the lungs.

Crampton and Haurowitz (1952) detected injected antigen at a maximum concentration in proteins from the lung of rabbits. Based on the data of Rapoport, cellular proliferates in the lungs were subsequently resorbed, leaving behind small sectors of fibrous swelling.*

Following the immunization of guinea pigs with anthrax vaccine, it was revealed that the process of immunogenesis, followed over a period of 7 months, began with the 1st day at the site of administration and continued up to 5 months in the regional lymph node, spleen and bone marrow. According to the literature (Vershilova, 1950; Korobkova, 1956; Yegoshin, 1960; Altareva et al., 1958; Vereninova et al., 1958; Borodko and Samsonovich, 1961), following vaccination with live mono- and polyvaccines immunity was preserved up to a year.

Minor changes, revealed by us in the heart, liver and kidneys, are fully conformable, since the vaccinal process is the reaction of the organism to the introduction of an attenuated infection, and though it

* The Greater Medical Encyclopedia, vol II, p 303.

is not accompanied by the development of the disease the entire organism takes part in the reaction, and immunity is created at the expense of a "minor illness" (Rapoport, 1965) or "adaptation through illness" (Davydovskiy, 1961).

Conclusions

1. During a histological study of the internal organs of experimental animals, vaccinated with the STI-1 and 54-F$_2$ strains, changes which are characteristic for anthrax were not revealed. Insignificant changes, detected in the liver, heart and kidneys of vaccinated animals, represented a reaction to the introduction of the antigen.

2. When rabbits and guinea pigs received the STI-1 and 34-F$_2$ vaccine strains, changes developed at the site of injection, the lymph nodes, spleen, bone marrow and lungs. These changes made it possible to judge the nature of the vaccinal process and its intensity.

3. During the macro- and microscopic investigation of tissue from the sites where the STI-1 and 54-F$_2$ vaccine strains were administered, it was established that the latter caused a significantly stronger local reaction, being expressed in an intensive leukocytic infiltration up to the formation of abscesses and necrosis of the muscle fibers of the subcutaneous tissue.

4. Following the vaccinations with both strains, the process of immunogenesis began with the 1st day (at the site of administration) and was preserved over a period of 5 months.

Literature

Altareva, N.D., Antonov, A. M., Zhdanov, V. M., et al., Trudy in-ta "Mikrob", Saratov, 1958, No 2, p 68.

Borocko, S. L., Samsonovich, L. G., In the book: Collection of Scientific Papers from the Elista Antiplague Station, Elista, 1961, No 2, p 221.

Vershilova, P. A., Zh. mikrobiol., 1950, No 6, p 3.

Vershilova, N. K., Kalacheva, N. F., Trudy in-ta "Mikrob", Saratov, 1958, No 2, p 1964.

Gavrilov, N. M., In the book: Collection of papers from the Student-veterinarian Meeting 1927--28, All-Union Institute of Experimental Veterinary Science, Moscow, 1929, vol 1, No 1, p 34.

Ginsburg, N. N., Kopylov, N. F., In the book: The STI Anthrax Vaccine, Moscow, 1946, p 194

Gurvich, G. A., Shumakova, G. V., Vestn. AMN SSSR, 1960, No 1, p57.

Idem., Byull. eksper. biol., 1957, No 10, p 95.

Davydovskiy, I. V., General Pathology of Man, Moscow, 1961.

Yegoshin, I. S., Thesis of Reports from the Inter-University Conference on the Pathological Anatomy of Agricultural Animals, Voronezh, 1960, p 19.

Rapoport, Ya., Greater Medical Encyclopedia, 1959, vol 11, p 298.

Idem., Arkh. pat., 1957, vol 2, p 3.

Idem., Pat. fiziol., 1965, No 4, p 8.

Figure 1. Plasmatization of cellular infiltrate at the site of administration of the 34-F_2 vaccine on the 14th day following vaccination.

Stained with hematoxylin-eosin. Magnification 665X.

Figure 2. Hyperplasia of bone marrow on the 2nd day following vaccination with the 34-F_2 strain.

Stained with hematoxylin-eosin. Magnification 665X.

GRAPHIC NOT REPRODUCIBLE

GRAPHIC NOT REPRODUCIBLE

Figure 3. Infiltration of the interalveolar septa by plasmadic cells on the 14th day after administration of the STI-1 vaccine strain.
Stained with hematoxylin-eosin.
Magnification 665X.

Figure 4. Fatty-basophilic degeneration of myocardium fibers of a rabbit, vaccinated with the 34-F_2 vaccine strain.
Stained according to Selye.
Magnification 500X.

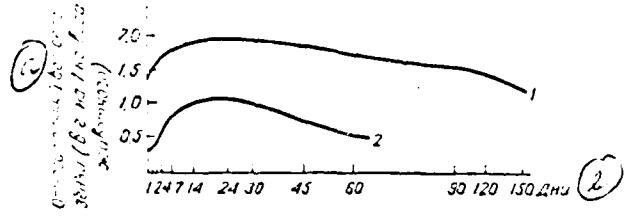


Figure 5. Dynamics of spleen weight for guinea pigs (1) and rabbits (2), vaccinated against anthrax.
a - relative weight of spleen (in grams per 1 kg of animal weight);
b - days.